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13. ABSTRACT (Maximum 200 words)

While it is well established that the SCN is the site of an endogenous circadian pacemaker that drives many behavioral and physiological rhythms, the output or coupling mechanism(s) for signaling the brain and the rest of the body is not known. We used an encapsulation technique to physically isolate the grafted neurons from the host brain. Because the donor period (about 24 hours) is easily distinguishable from the freerunning period (about 20 hours) of the host hamster, restored rhythms can be attributed unambiguously to the SCN of the donor tissue. Encapsulated SCN grafts (N=4) implanted into the 3rd ventricle of SCN-lesioned hamsters rescues wild-type locomotor behavior in (and only in) animals in which tissue survives within the capsule. This provides definitive evidence of diffusible output signals from the SCN in controlling locomotor rhythmicity.

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Final Technical Report: F49620-94-1-0294P0003**Investigator's Name:**

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I. a Statement of research objectives

The **original** objective of the present work was to determine whether there is a diffusible coupling signal from the biological clock, located in the SCN of the mammalian brain. We have now verified that such a signal exists in a limited group of 4 animals.

We are now continuing to optimize conditions for graft survival and development within the capsule. This entails an attack on 3 fronts. First, we have established optimal tissue harvesting procedures (see below). Second, optimal device (capsule) design must be determined. Third, optimal biocompatibility (graft device and host brain-device) conditions must be established.

Optimizing conditions for encapsulating the graft will permit subsequent development of materials with various molecular weight cutoffs, for determination of approximate molecular weight of the diffusible signal from the SCN.

A novel finding and a second objective emerged in the course of searching for procedures for optimizing the number of pacemaker cells in the graft. We discovered that a subset of SCN cells that are recognizable by virtue of the fact that they are immunopositive for calbindin (a calcium binding hormone) appeared to be necessary and sufficient for the restoration of circadian locomotor rhythmicity. This suggest 2 hypotheses: Either different circadian rhythms are regulated by different pacemaker cells; or pacemakers are restricted to a subnucleus of the SCN. These hypotheses are experimentally testable, and the

results will be important to the identification and characterization of SCN pacemaker cells and their specific function(s). This work was presented in the Toya Symposium (Japan) and will be presented at the Society for Neurosciences Meeting 1995.

I. b Status of the research.

The research to date indicates that there is a diffusible coupling signal from the SCN that is sufficient to regulate circadian rhythms of locomotor activity. We have now verified that such a signal exists in a limited group of 4 animals. The optimization of the tissue encapsulation procedures is proceeding on 3 fronts: optimal 1) harvesting of tissue, 2) device design, 3) biocompatibility of device. The first goal is met, the second goal is in progress and close to completion, the third goal remains to be done. Further details are provided below.

1c. Accomplishments/ New Findings

In the main series of studies in this proposal, we have used encapsulated SCN tissue to study the role of diffusible signals from the SCN in regulating locomotor rhythmicity. There has been tremendous interest in the use of encapsulation devices for providing biologically relevant signals in many different systems (e.g. pancreatic cells for treating diabetes, PC-12 cells secreting dopamine for treating Parkinsonism, adrenal medullary cells for treating pain in terminally ill cancer patients). Ours is the only group working on encapsulation of neural tissue.

We have taken fetal tissue from wild type donor hamsters and placed implants into tau mutant host animals. The results to date indicate that such encapsulated tissue - which can make no neural connections with the host brain - can restore circadian locomotor rhythmicity. Thus far, we have restored rhythmicity in 4 recipient animals. These results are definitive evidence for the presence of a diffusible signal from the SCN.

While the results are extremely encouraging, the low rate of encapsulated tissue survival is a problem. It appears that there is a problem in biocompatibility in that only about 4% of the animals recover. In most of the animals, the tissue in the capsule is dead at the time of sacrifice at 4-8 weeks after transplantation. The encapsulation technique must be to improved.

Improvement of the methodology will set the stage for identification of the diffusible factor. As described above, we are tackling the problem in a 3 fronted attack. First, we have improved tissue harvesting procedures, as follows. 1) We now use SCN punches

taken from vibratome sections, that have very little extra-SCN tissue for the donor tissue. Thus the capsule is filled with maximal numbers of pacemaker cells. 2) The neural tissue is implanted along with matrigel (contains a variety of growth factors) and with alginate (that isolates neural tissue within the capsule. This prevents the tissue from forming a "bolus" within the capsule, and allows a narrow "string" of tissue, which has access to nutrients and oxygen, essential for survival. Finally, we have developed a new procedure for extruding the tissue in a long "noodle", allowing us to fill the capsule completely.

Second, we have worked on device improvement and have made substantial progress in improving the hollow fiber material. The original capsule material was 15% PAN/PVC. The new material is 11% PAN/PVC and 4% PEO. 1) A major advantage of the new material is that one can see through it, allowing confirmation that the fiber is completely full prior to implantation. 2) The new material has improved water permeability, and improved flux both before and 3) following protein absorption at a full range of biologically relevant molecular weights. 4) The new device allows faster transport of materials across the membrane wall.

Anatomical studies indicate that the survival rate of tissue in the new capsules (at 8 weeks postimplantation) is about 70%, compared to about 10% for the older (PAN/PVC) materials. Behavioral studies on the efficacy of the new capsule materials in restoring locomotor rhythmicity are ongoing.

The final step in improving the encapsulated tissue methodology will be to minimize/eliminate problems of biocompatibility between host and device and graft and device. This work has not yet been done.

Our **second objective**, representing a new direction in the research, derives from the observation that very small tissue punches of the SCN are sufficient to regulate locomotor rhythmicity (a technique developed for the purpose of tissue harvesting for encapsulation of grafts). This work suggests that a subnucleus of the SCN that contains calcium binding cells (calbindin-D-28, CABP). We have made the following observations on the function of these cells. (1) Double-label immunocytochemistry indicates

that 60-80% of CaBP-ir cells express fos in animals exposed to light at CT 21, indicating that the CaBP cells receive retinal input. (2) In ablation studies, partial lesions that spared the CaBP subnucleus of the SCN did not abolish locomotor activity rhythms. In contrast, lesions that destroyed the CaBP cells disrupted rhythmicity, even when vasopressin and vasoactive intestinal polypeptide cells and fibers were spared. This suggests a role for the CaBP subnucleus in circadian organization. (3) We then compared the number of SCN CaBP cells between wild type ($\tau=24h$) and mutant ($\tau=20h$) hamsters kept in constant darkness. Homozygote mutant hamsters have more CaBP cells than do wild type hamsters. This is the first description of a phenotypic difference between wild type and τ mutant hamsters. (4) Of the transplants that restored donor specific rhythmicity, some contained VIP, and all contained NP and CaBP. Among the SCN grafts that did not restore rhythmicity, most had both NP and VIP, some had NP and no VIP, while none contained SCN CaBP cells. This suggests localization of pacemaker cells controlling circadian locomotor in the CaBP subnucleus of the SCN. The availability of this new marker disputes the previously held notion that all SCN cells are equipotential in their oscillator function.

Personnel Supported:

Joseph LeSauter Research Associate

Rae Silver Professor (course release time)

Publications

LeSauter, J., Lehman, M.N., Silver, R. Restoration of circadian rhythmicity by transplants of SCN "micropunches" (submitted).

Silver, R. (Rapporteur) Written in collaboration with Drs. G. Block-UVA, M.Zatz-NIH, M.Lehman- U Cincinnati, T. Van den Pol-Yale U., T. Roenneberg, Germany. Edited by J. Dunlap and J. Loros Cellular basis of clocks.

Lehman, M., LeSauter, J., Kim, C., Berryman, S., Tresco, P., Silver, R. (1995) How do fetal grafts of the SCN communicate with the host brain? Cell Transplantation 4: 75-81.

Serviere, J., Gendrot, G. LeSauter, J. and R. Silver (1994). Host resets phase of grafted SCN: I. A 2-deoxyglucose study of the time course of entrainment. Brain Res. 665: 168-176.

- Serviere, J., Gendrot, G. LeSauter, J. and R. Silver (1994). Host resets phase of grafted SCN: II. Influence of implant site, tissue specificity, and pineal secretions. *Neurosc. Lett.* 176: 80-84.
- LeSauter, J. and Silver, R. (1994) Suprachiasmatic nucleus lesions abolish and fetal SCN grafts restore circadian gnawing rhythms in hamsters. *Restorative Neurol. and Neurosc.*: 6: 135-143.
- Lehman, M.. and R. Silver (1994). Restoration of circadian rhythms by neural transplants. *Neuronal Transplantation, CNS Neuronal Injury and Regeneration.* (Eds. J. Marwah, P. Teitelbaum, and K.N. Prasad.) CRC Press Inc. pp. 141-160.

Interactions/Transitions

a. Papers presented in 1994-95

- Silver, R., LeSauter, J. and Lehman, M. (1995). Localization of pacemaker cells in the hamster SCN: I) Studies using fos, ablation and mutants. Soc. Neurosc. Abstr.
- Silver, R., LeSauter, J. and Lehman, M. (1995). Localization of pacemaker cells in the hamster SCN: II) Transplant studies. Soc. Neurosc. Abstr.
- Silver, R., (8/95) Diffusible signals of the SCN. Honma Conference, Sapporo, Japan.
- Silver, R. and LeSauter (8/95) Localization of pacemaker cells in the hamster SCN. Lake Toya Conference, Japan.
- Lehman, M. LeSauter, J. Kim, C. and Silver, R. (1995) Evidence suggesting migration of host neurons into fetal anterior hypothalamic grafts. Soc. Neurosc. Abstr.
- Silver, R. (7/95) Cellular basis of clocks. American Physiological Society. Dartmouth.
- Silver, R. (4/95) Encapsulated SCN grafts. Keystone Symposium, Colorado
- LeSauter J., P. Romero, and R. Silver (1994). Location of SCN graft influences precision of recovered circadian rhythm. Soc. Res. Biol. Rhythms Abstr. 4 34.
- Romero, M.T., J. LeSauter, P. Romero, M.. Lehman, and R. Silver (1994). Restoration of function: Principles derived from studies of suprachiasmatic nucleus (SCN) transplants. Am. Soc. Neural Transplantation Abstr. 1:12.

- Lehman, M.N., J. LeSauter, C. Kim, S.J. Berryman, P. Tresco, and R. Silver (1994). How do grafts of the suprachiasmatic nucleus communicate with the host brain? Am. Soc. for Neural Transplantation Abstr. 1:12.
- Romero, P., J. LeSauter, and R. Silver (5/1994). Distance of SCN graft from lesion site influences precision of recovered circadian activity rhythm. New England Consortium for Undergraduate Science Education (NECUSE). Student Workshop on photoperiodism, rhythms and clocks.
- M.D. Cascio, J. Le Sauter, and R. Silver (5/1994). Intact vs. grafted SCN: comparison of VIP and VP-ergic fiber projections. New England Consortium for Undergraduate Science Education (NECUSE). Student Workshop on photoperiodism, rhythms and clocks.
- Silver R., J. Le Sauter, C. Kim, M. Lehman (5/1994). How do fetal grafts of the suprachiasmatic nucleus communicate with the host brain? 5th International Symposium on Neural Transplantation.

b. Consulting and advisory functions

- 1995 National Science Foundation, Committee of Visitors 6/95
- 1994-1996: NIMH Psychobiology, Behavior, and Neurosciences Panel Member
- 1993-1994: Panel Member, NIMH Behavioral Neurosciences
- 1995: American Physiological Society, Conference on "Understanding the Biological Clock - from Genetics to Physiology", Rapporteur.
- 1994-1999: Associate Editor, Journal of Biological Rhythms
- 1994-1997: Advisory Committee, General Clinical Research Center, University of Virginia, Department of Medicine
- 1994-1996: Member-at-Large: Soc. Res. Biol. Rhythms
- 1994: Site visit panel member: NIH review of program project grant at Morehouse School of Medicine
- 1995-1999: Editorial Board: Journal for Research in Biological Rhythms-

c. Transitions

Dr. Patrick Tresco, my collaborator in the Department of Engineering at the University of Utah, visited Dr. Patrick Aebischer. (Dr. Aebischer is the individual often credited for the introduction of the polymer capsule; see Scientific American, June 1995) idea in the 1980's. When Aebischer was at Brown University, I began the collaboration with him and with his then Ph.D. student, Patrick Tresco. Aebischer moved back to Switzerland, and I have continued the work with Tresco (now at University of Utah). At his hospital and laboratory in Lausanne, Switzerland, Dr.

Aebischer is engaged in the first human clinical trials using PAN/PVC for encapsulation of adrenal tissue in the spinal cord for treatment of pain in terminally ill cancer patients. He was extremely interested in our data on encapsulated neural tissue, and plans to incorporate the results into his work.

If New Discoveries/Inventions

None